## AMENDMENTS

Please enter the following amendments into the specification as filed:

On page 1, lines 3-7:

This application is a divisional application of U.S. Serial No. 09/315,114, filed May 19, 1999, now U.S. Patent No. 6,632,399, issued October 14, 2003, which is a continuation-in-part of ~~claims priority to~~ U.S. Serial No. 09/083,678, filed May 22, 1998, now U.S. Patent No. 6,063,589, issued March 15, 2000, which is related to U.S. Serial No. 60/047,488, filed May 23, 1997. ~~This application is also related to U.S. Serial No. 08/995,056, filed December 19, 1997, U.S. Serial No. 08/910,726, filed August 12, 1997, U.S. Serial No. 08/768,990, filed December 19, 1996 and U.S. Serial No. 08/761,036, filed December 5, 1996,~~ the disclosures of each of which are explicitly incorporated by reference herein.

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**Page 2, lines 19-21**

U.S. Patent No. 5,160,702, issued November 3, 1993 to Kopf-Sill and Zuk discloses rotational frequency-dependent "valves" using capillary forces and siphons, dependent on "wettability" of liquids used to prime said siphon.

**Page 3, lines 8-9**

Renoe *et al.*, 1974 *Clin. Chem.* 20: 955-960 teach a "minidisc" module for a centrifugal analyzer.

**Page 8, line 29 to page 9, line 4**

For the purposes of this invention, the term "biological sample" or "biological fluid sample" will be understood to mean any biologically-derived analytical sample, including but not limited to blood, plasma, serum, lymph, saliva, tears, cerebrospinal fluid, urine, sweat, plant and vegetable extracts, semen, cell culture fluids, cellular lysate, aqueous or non-aqueous fractions of the above and ascites fluid.

**Page 11, line 29 to page 13, line 12**

Platforms of the invention such as disks and the components comprising such platforms are advantageously provided having a variety of composition and surface coatings appropriate for a particular application. Platform composition will be a function of structural requirements, manufacturing processes, and reagent compatibility/chemical resistance properties. Specifically, platforms are provided that are made from inorganic

crystalline or amorphous materials, *e.g.* silicon, silica, quartz, inert metals, or from organic materials such as plastics, for example, poly(methyl methacrylate) (PMMA), acetonitrile-butadiene-styrene (ABS), polycarbonate, polyethylene, polystyrene, polyolefins, polypropylene and metallocene. These may be used with unmodified or modified surfaces. Surface properties of these materials may be modified for specific applications. Surface modification can be achieved by silanization, ion implantation and chemical treatment with inert-gas plasmas (*i.e.*, gases through which electrical currents are passed to create ionization). Also provided by the invention are platforms made of composites or combinations of these materials, *for example*, platforms manufactured ~~manufactures~~ of a plastic material having embedded therein an optically transparent glass surface comprising for example the detection chamber of the platform. Microplatform disks of the invention are preferably fabricated from thermoplastics such as teflon, polyethylene, polypropylene, methylmethacrylates and polycarbonates, among others, due to their ease of molding, stamping and milling. Alternatively, the disks can be made of silica, glass, quartz or inert metal. A fluid handling system is built by sequential application of one or more of these materials laid down in stepwise fashion onto the thermoplastic substrate. Alternatively, the entire disc can be injection molded, embossed or stamped. Disks of the invention are fabricated with an injection molded, optically-clear base layer having optical pits in the manner of a conventional compact disk (CD). The optical pits provide means for encoding instrument control programming, user interface information, graphics, data analysis, and sound specific to the application and driver configuration. The driver configuration depends on whether the micromanipulation device is a hand-held, benchtop or floor model, and also on the details of external communication and other specifics of the hardware configuration. This layer is then overlaid with a reflective surface, with appropriate windows for external detectors, specifically optical detectors, being left clear on the disk. Other layers of polycarbonate of varying thickness are laid down on the disk in the form of channels, reservoirs, reaction chambers and other structures, including provisions on the disk for valves and other control elements. These layers can be pre-fabricated and cut with the appropriate geometries for a given application and assembled on the disk. Layers comprising materials other than polycarbonate can also be incorporated into the disk. The

composition of the layers on the disk depend in large part on the specific application and the requirements of chemical compatibility with the reagents to be used with the disk. Electrical layers can be incorporated in disks requiring electric circuits, such as electrophoresis applications and electrically-controlled valves. Control devices, such as integrated circuits, laser diodes, photodiodes and resistive networks that can form selective heating areas or flexible logic structures can be incorporated into appropriately wired recesses, either by direct fabrication of modular installation onto the disk. Reagents that can be stored dry can be introduced into appropriate open chambers by spraying into reservoirs using means similar to inkjet printing heads, and then dried on the disk. A top layer comprising access ports and air vents, ports or shafts is then applied. Liquid reagents are then injected into the appropriate reservoirs, followed by application of a protective cover layer comprising a thin plastic film.

**Page 13, line 26 to page 14, line 25**

The platforms of the invention are provided comprising microfluidics handling structures in fluidic contract with one another. In preferred embodiments, fluidic contact is provided by capillary or microchannels comprising the surface of the platforms of the invention.. Microchannel sizes are optimally determined by specific applications and by the amount of delivery rates required for each particular embodiment of the platforms and methods of the invention. Microchannel sizes can range from 0.02mm to a value close to the thickness of the platform. Microchannel shapes can be trapezoid, circular or other geometric shapes as required. Microchannels preferably are embedded in a platform having a thickness of about 0.1 to 100mm, wherein the cross-sectional dimension of the microchannels across the thickness dimension of the platform is less than 500μm -800μm and from 1 to 90 percent of said cross-sectional dimension of the platform. In these embodiments, which are~~is~~ based on the use of rotationally-induced fluid pressure to overcome capillary forces, it is recognized that fluid flow is dependent on the orientation of the surfaces of the components. Fluids which completely or partially wet the material of the microchannels, reservoirs, detection chambers, etc. (*i.e.*, the components) of the

platforms of the invention which contain them experience a resistance to flow when moving from a component of narrow cross-section to one of larger cross-section, while those fluids which do not wet these materials resist flowing from components of the platforms of the invention of large cross-section to those with smaller cross-section. This capillary pressure varies inversely with the sizes of the two components, or combinations thereof, the surface tension of the fluid, and the contact angle of the fluid on the material of the components . Generally, the details of the cross-sectional shape are not important, but the dependence on cross-sectional dimension results in microchannels of dimension less than 500μm exhibit significant capillary pressure. By varying the intersection shapes, materials and cross-sectional areas of the components of the platform of the invention, "valves" are fashioned that require the application of a particular pressure on the fluid to induce fluid flow. This pressure is applied in the disks of the invention by rotation of the disk (which has been shown above to vary with the square of the rotational frequency, with the radial position and with the extent of the fluid in the radial direction). By varying capillary valve cross-sectional dimensions as well as the position and extent along the radial direction of the fluid handling components of the platforms of the invention, capillary valves are formed to release fluid flow in a rotation-dependent manner, using rotation rates of from 100rpm to several thousand rpm. This arrangement allows complex, multistep fluid processes to be carried out using a pre-determined, monotonic increase in rotational rate.

**Page 17, lines 8-22**

As described herein for performing blood glucose assays (and as understood in the art that essentially the same microfluidics structures can be used for a multiplicity of blood analyte assays or, more generally, for analyte assays in any fluid sample, most preferably a biological fluid sample), a capillary barrier prevents movement of the fluid sample directly into the assay chamber **107**. In the metering structure shown in Figure 1A, fluid (or more properly for this illustrative example, blood) metering capillary **102** acted as a capillary barrier that prevented blood fluid flow from metering capillary **102** at

a first, non-zero rotational speed $f_1$, ranging from about 200rpm to about 450rpm and sufficient to permit fluid flow comprising overflow from the entry port **101** through overflow capillary **121** and into overflow chamber **122**. This capillary boundary was constructed to be overcome at a second rotational speed $f_2$, ranging from about 250rpm to about 900rpm (where $f_2 > f_1$). In the alternative embodiments shown in Figures 1B and 1C, blood fluid chamber **104** acted as a capillary barrier that was maintained during rotation at a rotational speed sufficient to ~~motivated~~ motivate excess fluid sample from the entry port **101** to the overflow chamber **122**, and was overcome at a second rotational speed greater than the first rotational speed to permit fluid sample flow into assay chamber **107**.

**Page 21, lines 4-20**

As blood flows into depression **113**, the fluid component of the blood is driven by pressure and hydrophilic forces into matrix **106**; the pore size of the matrix is chosen to prevent the cellular components of the blood from entering the matrix (Figure 1.4). In preferred embodiment, the cellular component of the blood is retained in depression **113** and the fluid component is efficiently distributed by wicking and by centripetal force into matrix **106**. In an alternative embodiment, alternative matrix **118** comprises at least two distinct elements that are compressed or adhered to one another in the assays chamber. The first element is similar to the reagent-containing matrix **106** described above; however, this embodiment of the matrix has a pore size that is not limited by the size of cellular components of the blood, and can be any pore size deemed optimal on experimental, economic, manufacturing or availability grounds. The second element comprises a filtering layer having a pore size that prevents cells and cellular debris from entering this portion of the matrix. In a preferred embodiment, the two elements are ~~and~~ rearranged in assay chamber **107** so that second matrix element is in contact with depression **113** wherein the blood aliquot is first contacted with the second matrix component. Blood fluid flows into and through the second matrix element and into the first matrix element, whereby cellular components of blood are prevented from entering

assay chamber **107** by the pore size of second matrix element. Preferably, the dimensions of the matrix element is about 1cm by about 0.75cm and has a thickness of about 0.05cm.

**Page 24, line 24 to page 25, line 7**

At a rotational speed $\mathbf{f_2}$ of about 100-1000rpm, the precise amount of fluid contained in metering capillary **202** is delivered into assay chamber **207**. In embodiments comprising a sacrificial valve **211** in-line with capillary **210** at a position between capillary **210** and assay chamber **207** ~~as shown in Figure 3.1~~, release of the sacrificial valve results in fluid flow through capillary **212** and into assay chamber **207**. In said embodiments, fluid flow is achieved at rotational speed $\mathbf{f_2}$ with removal of the sacrificial valve. In embodiments of the platforms of the invention comprising capillary valve **211** at a position between capillary **210** and **212** ~~as shown in Figure 3B~~, capillary **210** preferably fills along with filling of blood metering capillary **202** until blood reaches capillary junction **211** at the junction between capillary **210** and capillary **212**; in such embodiments, the capillary junction had a depth of from about 0.03mm to about 2.2mm. At a rotational speed $\mathbf{f_2}$ of about 100-1000rpm, the fluid contained in blood metering capillary **202** is delivered into assay chamber **207** (Figure ~~3~~4C).

**Page 26, lines 1-24**

After a time sufficient to produce a detectable amount of a colored product, the wash buffer is released from wash buffer reservoir **223** through capillary **222** and into assay chamber **207**. In embodiments comprising a sacrificial valve **225** in-line with capillary **222** at a position between capillary **222** and assay chamber **207** ~~shown in Figure 3.3~~, release of the sacrificial valve results in fluid flow through capillary **222** and into assay chamber **207**. In said embodiments, fluid flow is achieved at rotational speed $\mathbf{f_3}$ of about 250rpm to about 1500rpm with removal of the sacrificial valve. In embodiments of the platforms of the invention comprising capillary valve **224** at a position between capillary **222** and assay chamber **207** ~~shown in Figure 3D~~, capillary **222** preferably fills

along with filling of blood fluid chamber **204** until blood reached capillary junction **224**; in such embodiments, the capillary junction had a depth of from about 0.03mm to about 2.2mm. At a higher rotational speed $f_4$ of about 400-2000rpm, the fluid contained in wash reservoir **223** is delivered into assay chamber **207** (Figure ~~3~~4D). Because the fluid flow of wash buffer into the assay chamber and fluid from the assay chamber to the waste chamber is laminar, there is very little mixing of the washing fluid with the fluid initially in the assay chamber. The wash fluid displaces the fluid sample in the assay by pushing it into the waste chamber. The exit of capillary **220** into chamber **221** is at a radial position such that assay chamber **207** must remain filled with fluid during this washing process. The quality of fluid removal is such that no more than 1 part in 1000 of the fluid in the chamber **207** (which has not been imbibed into matrix **206**) remains. The fluid which has wicked into matrix **206** is not removed during this wash because of the small pore size of the matrix which resists fluid flow; furthermore, color reagents do not diffuse out of matrix **206** if the wash time is relatively short (less than a few hundred seconds). As a result, interfering blood fluid components such as hemoglobin are removed from chamber **207** while substantially leaving behind color reagents in matrix **206**. These are then interrogated spectrophotometrically in assay chamber **207** ~~(Figure 4E)~~.

**Page 31, line 26 to page 32, line 10**

Blood fluid exits cell separation chamber **407** through capillary **420** and flows into assay chamber **421** (Figure 6D), with displaced air flowing through air channel **414**, and may be vented to the surface of the disc or in communication with cell separation chamber **407** ~~(Figure 5.4)~~. Blood fluid flows into assay chamber **421** and wicks into matrix **422**. As the blood fluid wicks into matrix **422**, dried reagents are solubilized and the reaction of the blood component catalyzed by said reagents proceeds as described below. The timescale over which these reactions take place is chosen to be long compared with the time it takes for the fluid component of blood to saturate the matrix **422**; however, in preferred embodiments, the reaction(s) goes to completion within about 0.5 min to about 5min. Reaction of the blood component(s) with reagents produce a

colored product (Figure 6E). In preferred embodiments, detection of colored product is performed spectrophotometrically, although visual inspection is also contemplated in alternative embodiments of the invention. The amount of colored product produced is detected and the amount of glucose in the blood sample determined thereby.

**Page 32, lines 11-13**

Another alternative embodiment of a blood glucose assay microsystems platform is shown in Figures 7A -7E~~D~~. Construction of the disk embodiments of the platforms of the invention were as described above.

**Page 37, line 22 to page 38, line 5**

As illustrated in Figures 8A through 8E~~D~~, in the use of this platform a volume of blood is applied to metering capillary **602**, either directly or using the metering components of the platform described above. Blood flows into cell separation chamber **607** with displaced air flowing through air channel **654**, and may be vented to the surface of the disc or in communication with blood fluid chamber **604** (Figure 8B). As blood flows into cell separation chamber **607**, the fluid component of the blood wicks into matrix **606**; the pore size of the matrix (from about 0.2μm to about 2μm) is chosen to prevent the cellular components of the blood from entering the matrix. In a preferred embodiment, matrix **606** is arranged in cell separation chamber **607** so that the matrix is in contact with or more preferably adhered to the lower surface of cell separation chamber **607**. Blood fluid, such as plasma or serum, traverses matrix **606** by wicking and under rotation-induced pressure, saturating the matrix and filling a space formed between the top surface of the matrix and the top surface of cell separation chamber **607** (Figure 8C).

**Page 38, lines 6-21**

Blood fluid exits cell separation chamber **607** through capillary **620~~610~~** and flows into mixing chamber **615**. Similarly, at a rotational speed of from about 200rpm to about 2000rpm sufficient to overcome capillary valve **625**, or upon release of sacrificial valve **625**, solubilized reagents **608** flow through capillary **620** and into mixing chamber **615** ~~(Figure 8C)~~. Fluid flow within mixing chamber **615** is turbulent, in contrast to fluid flow through capillaries **610** and **620**, which is primarily laminar, so that mixing occurs predominantly in mixing chamber **615**. Fluid flow proceeds through channel **636** and then either through second mixing chamber **640** or directly through capillary **646** into mixed fluid receiving chamber **637** (Figure 8D).

~~Glucose detection reagents mixed with blood~~ Reaction of the blood component(s) with reagents **608** produce a colored product (Figure 8E) in the mixed fluid receiving chamber **637** ~~(Figure 8D)~~. The timescale over which these reactions take place preferably goes to completion within about 0.5 min. to about 5min. ~~Reaction of the blood component(s) with reagents 608 produce a colored product (Figure 8D).~~ Detection of the colored product of the glucose detecting reaction is performed in mixed fluid receiving chamber **637**. In preferred embodiments, detection is performed spectrophotometrically, although visual inspection is also contemplated in alternative embodiments of the invention. The amount of colored product produced is detected and the amount of glucose in the blood sample determined thereby.

**Page 41, lines 3-15**

In an alternative reaction protocol, glucose oxidase is used to produce hydrogen peroxide by oxidation of glucose in the blood sample. In this reaction scheme, glucose oxidase converts glucose to gluconic acid and hydrogen peroxide; a twice-stoichiometric amount of hydrogen peroxide is produced relative to the amount of glucose present in the blood sample. The hydrogen peroxide then oxidizes a dye precursor present in the assay chamber or preferably within a detection cell, yielding a colored product. A variety of

dye precursors are useful in the practice of this aspect of the invention, including but not limited to O-dianisidine, O-toluidine, O-tolidine, benzidine, 2,2'-azinodi-(3-ethylbenzthiazoline sulfonic acid), 3-methyl-2-benzthiazolinone hydrazone plus N,N-dimethylaniline, phenol~~phenyl~~ plus 4-aminophenzanone, sulfonated 2,4-dichlorophenol plus 4-aminophenzanone, 3-methyl-2-benzothiazolinone hydrazone plus 3-(dimethylamino) benzoic acid, 2-methoxy-4-allyl phenol and 4-aminoantipyrene-dimethylaniline. Optimum reagent component concentration vary according to the specifics of the application of this chemical assay, as well understood and practiced by one versed in the art. This reaction scheme is illustrated as follows:

**Page 45, lines 3-11**

As illustrated in Figure 9~~A~~, in the use of this platform a volume of blood from about 15μL to about 150μL is applied to metering capillary **702**, either directly or using the metering components of the platform described above. Blood flowing through capillary **710** and lysis buffer flowing through capillary **718** are mixed in mixing chamber **715** by overcoming capillary valve **711** or release of sacrificial valve **711**. A volume of lysis buffer from about 25μL to about 90μL was mixed with the blood sample. Fluid flow within mixing chamber **715** is turbulent, in contrast to fluid flow through capillaries **710** and **718**, which is primarily laminar, so that mixing occurs predominantly in mixing chamber **715**. Fluid flow proceeds through channel **717** and into secondary metering structure **719**.

**Page 46, line 24 to page 47, line 6**

Metering capillary **802** is fluidly connected to capillary **810** that is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm. Capillary **810** is further fluidly connected to mixing chamber **815** that is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about 10cm, and is positioned from about 1.2cm to about 14cm from the

center of rotation. Lysis buffer chamber **816** containing blood lysis buffer is from about 0.02mm to about 3cm deep, has a cross-sectional diameter of from about 0.02mm to about10cm, and is positioned from about 1.2cm to about 14cm from the center of rotation. Lysis buffer chamber **816** is positioned more proximally to the axis of rotation thanthat mixing chamber **815**, and has a volumetric capacity of from about 15μL to about 150μL of lysis buffer, composed of 0.1% Triton X100 in 50mM Tris pH 9.5. Lysis buffer chamber **815** is fluidly connected through capillary **818** to mixing chamber **815**.

**Page 48, lines 21-26**

Boronate affinity matrix chamber **822** is further fluidly connected to capillary **832**. Capillary 832~~732~~ is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm. Capillary **832** is further fluidly connected with waste reservoir **834**. Waste reservoir **834** is from about 0.02mm to about 3cm deep and has a cross-sectional diameter of from about 0.02mm to about 10cm and is positioned from about 1.2cm to about 14cm from the axis of rotation.

**Page 51, lines 14-26**

Capillary **918** is further fluidly connected to secondary metering structure **919**. Secondary metering structure **919** is from about 0.02mm to about 3cm deep, and is positioned from about 1.2cm to about 14cm from the center of rotation. Secondary metering structure **919** is constructed to comprise three sections. A first section comprises a throwaway section sample having a volumetric capacity of from about 5μL to about 10μL because it is thought that by taking the second section a more representative sample would be obtained. This throwaway section is arranged proximal to the entry position of capillary **918** and is separated from a metering section by a septum that extends from the distal wall of the structure to a position just short of the proximal wall of the structure. This arrangement produces a fluid connection between

the throwaway section and the metering section. The metering section has a volumetric capacity of from about 5μL to about 10μL and is fluidly connected to an overflow section having an excess volumetric capacity of from about 15μL to about 150μL. The volumetric capacity of the overflow section is sufficient to accommodate the largest blood fluid volume applied to the disk.

**Page 53, line 25 to page 54, line 28**

As illustrated in Figure 11, in the use of this platform a volume of blood from about 15μL to about 150μL is applied to metering capillary **902**, either directly or using the metering components of the platform described above (Figure 11A). Release of sacrificial valve **911** and rotation of the platform at a rotational speed $f_1$ of from about 50rpm to about 1000rpm motivates blood flow through capillary **910** and into blood lysis chamber **915** (Figure 11C). The mixture of blood and blood lysis buffer in blood lysis chamber **915** is mixed by agitation, wherein the platform is accelerated repeatedly from about +2000rpm/sec to -2000rpm/sec (wherein "+" and "-" indicate rotation in different directions) over a time period of about 30sec to about 600sec (Figure 11D and 11E). Release of sacrificial valve **913** and rotation of the platform at a rotational speed $f_2$ of from about 200rpm to about 2000rpm motivates the lysed blood sample to flow through capillary **918** and into secondary metering structure **919** (Figure 11F). Continued rotation motivates lysed blood solution to fill the metering section of secondary metering structure **919**; after filling of this section of the structure, excess lysed blood sample flows through capillary **941** and into total hemoglobin read chamber **942**. After filling of total hemoglobin read chamber **942**, any excess lysed blood sample is displaced into the overflow section of secondary metering structure **919**.

Release of sacrificial valve **923** and rotation of the platform at a rotational speed $f_3$ of from about 200rpm to about 2000rpm motivates the metered lysed blood sample in the metering section of secondary metering structure **922** to flow through capillary **921** and into boronate affinity matrix chamber **922** (Figure 11G). After incubation of the metered lysed blood sample **956** in boronate affinity matrix chamber **922**, sacrificial

valve **936** is released and the platform is rotated at a rotational speed $f_4$ of from about 200rpm to about 3000rpm. Column wash buffer flows from column wash buffer reservoir **929** through capillary **921** and into boronate affinity matrix chamber **922**, displacing the unbound hemoglobin fraction through capillary **932** and into sample collection cuvette array **934** (Figure 11H). Continued rotation of the platform displaces the collected sample sequentially into the separate cuvettes radially away from the position of boronate affinity matrix chamber **922** on the platform (Figure 11J). Sample collection cuvette array **934** is then interrogated by illumination with light at a wavelength of 430nm, and the concentration of non-glycated hemoglobin in the blood sample determined. Additionally, illumination of total hemoglobin read chamber **942** with light at a wavelength at 415nm is performed to determine the concentration of total hemoglobin in the blood sample. The amount of glycated hemoglobin is calculated by subtracting the amount of non-glycated hemoglobin in the sample from the total hemoglobin concentration in the sample. Control sample read cuvettes **943** and **944** are used to calibrate the spectrophotometric readings.

**Page 55, lines 12-25**

A second alternative embodiment of the glycated hemoglobin microsystems assays of the invention comprises inositol hexaphosphate. In these embodiments, inositol hexaphosphate is attached (covalently or by electrostatic interactions) to a solid support, including but not limited to beads, membranes, pads, etc. The lysed blood sample is treated with sodium dithionite to convert it to the deoxy form. In the microsystems platforms of the invention, an effective amount of sodium dithionite is provided with the lysis buffer, or as a component of the secondary metering structures, most preferably as a dry powder coating on the walls of one or both of the metering sections thereof. The deoxygenated lysed blood sample is then placed in contact with the solid support comprising inositol hexaphosphate, preferably comprising and in substitution for the boronate affinity matrix chamber of the glycated hemoglobin platforms of the invention. In these embodiments, the portion of hemoglobin that does not bind to the inositol

phosphate-containing solid support is the glycated fraction, which can be delivered to a readreads chamber, cuvette or other optically-appropriate component of the platform and the amount of glycated hemoglobin determine directly by visible light reflectance spectrophotometry at a wavelength of 415nm.

**Page 60, lines 1-15**

Boronate affinity matrix chamber **28** is further fluidly connected to capillary **37**. Capillary **37** is from about 0.02mm to about 2mm deep and has a cross-sectional diameter of from about 0.02mm to about 2mm and is connected to sample collection cuvette array **12**. Sample collection cuvette array **12** is from about 0.02mm to about 3cm deep and has a cross-sectional diameter of from about 0.02mm to about 10cm and is positioned from about 1.2cm to about 14cm from the axis of rotation. Sample collection cuvette array **12** is separated into a multiplicity of individual chambers, each separated from one another by septa that extend from the distal wall of the cuvettes to a position adjacent to the proximal wall of the cuvettes, so that a fluid passage **50** is maintained between each of the cuvettes. The fluid passage **50** is formed by the back (proximal wall) of the sample collection cuvette array **12** and the row of septa separating each of the sections of the sample collection cuvettes **12**. Capillary 3733 is fluidly connected to sample collection cuvette array **12** at a position adjacent to the proximal wall of the array and directed to the cuvette most proximal to the boronate affinity matrix chamber **28**. In alternative embodiments, collection cuvette array **12** can be constructed without such septa, and this structure is then just a single collection chamber.

**Page 62, lines 10-18**

The disc is then accelerated to rotational speed $f_3$ of about 500rpm to about 3000rpm, typically about 1000rpm, with release of sacrificial valve **7A** and fluid flow of from about 1μL to about 50μL, typically about 5μL of blood from metered subvolume **2** through capillary **7** and into blood lysis chamber **16** containing from about 25μL to about

90μL, typically about 45μL of blood lysis buffer. This is shown in Figure 12H. The mixture of blood and blood lysis buffer in blood lysis chamber **16** is mixed by agitation, wherein the platform is accelerated repeatedly from about +2000rpm/sec to -2000rpm/sec (wherein ⊟+⊟ and ⊟–⊟ indicate rotation in different directions), typically from about 250-500rpm/sec, over a time period of about 30 seconds to about 5min, typically 1-2min, as shown in Figures 12I and 12J.

**Page 63, lines 8-19**

The disc is then accelerated to a rotational speed $f_2$ from about 500rpm to about 3000rpm and typically about 1000rpm, and sacrificial valve **32** is released. A volume of about 250μL to about 350μL, typically about 290μL of column wash buffer as described above flows from wash buffer reservoir **30** though capillary **31** and into boronate affinity matrix chamber **28** (shown in Figure 12O). The wash buffer displaces the non-glycated hemoglobin and other components of the lysed blood fluid from the affinity column matrix and into sample collection cuvette array **12**. Figures 12P through 12Q show sequential filling of the individual cuvettes in sample collection cuvette array **12**. The rotation speed of the disc is reduced, to from about 0rpm to about 500rpm and typically to about 60rpm for sample collection cuvette array **12** and total hemoglobin read chamber **23** to be interrogated spectrophotometrically. The glycated fraction of the blood sample is determined~~determine~~ algorithmically by subtracting the non-glycated hemoglobin fraction in sample collection cuvette array **12** from the total hemoglobin detected in total hemoglobin read chamber **23**.

**Page 65, line 18 to page 66, line 8**

The ability to specifically generate heat at a particular location on a microsystems platform of the invention also enables the use of sacrificial valves that can be released or dissolved using heat. For the purposes of this invention, the term "⊟sacrificial valve"⊟ is intended to encompass materials comprising waxes, plastics, and other material that

can form a solid or semi-solid fluid-tight obstruction in a microchannel, capillary, chamber, reservoir or other microfluidics component of the platforms of the invention, and that can be melted or deformed to remove the obstruction with the application of heat. Sacrificial valves are preferably made of a fungible material that can be removed from the fluid flow path. In preferred embodiments, said sacrificial valves are wax valves and are removed from the fluid flow path by heating, using any of a variety of heating means including infrared illumination and most preferably by activation of resistive heating elements on or embedded in the platform surface as described herein. For the purposes of this invention, the term "wax" is intended to encompass any solid, semi-solid or viscous liquid hydrocarbon, or a plastic. Examples include mondisperse hydrocarbons such as eicosane, tetracosane and octasone, and polydisperse hydrocarbons such as paraffin. In the use of wax sacrificial valves, application of a temperature higher than the melting temperature of the wax melts the valve and removes the occlusion from the microchannel, capillary or other fluidic component of the microsystems platforms of the invention. Particularly when the sacrificial valve is melted on a rotating microsystems platform of the invention, the melted wax to flow through the microchannel, capillary or other fluidic component of the microsystems platforms of the invention and away from the original site of the valve.

**Page 66, lines 9-26**

One drawback, however, is the possibility that the wax will recrystallize as it flows away from the original valve site, and concomitantly, away from the localized heat source. Recrystallization results in re-occlusion of the microchannel, capillary or other fluidic component of the microsystems platforms of the invention, potentially and most likely at a site other than the site of a localized heat source, and therefore likely to foul fluid movement on the disc. One solution for this problem is the inclusion in the sacrificial wax valves of the invention of a wax recrystallization chamber positioned downstream from the position of the wax valve. Preferably, the wax recrystallization chamber is fluidly connected with the microchannel, capillary or other fluidic component

of the microsystems platforms of the invention that was occluded by the wax sacrificial valve. Typically, the wax recrystallization chamber is a widening of the microchannel, capillary or other fluidic component of the microsystems platforms of the invention so that recrystallized wax can harden on the walls of the microchannel, capillary or other fluidic component of the microsystems platforms of the invention with enough distance between said walls that the recrystallized wax does not re-occlude the microchannel, capillary or other fluidic component of the microsystems platforms of the invention. Preferably, the heating element, most preferably the resistive heating element of the invention, extends past the site of the wax valve and overlaps at least a portion of the wax recrystallization chamber, thereby retarding the propensity of the wax valve to recrystallize.

**Page 66, line 27 to page 67, line 21**

It is also recognized that this propensity of wax valves to recrystallize can be exploited to create a wax valve at a particular location in a microchannel, capillary or other fluidic component of the microsystems platforms of the invention. In this embodiment, a particular location can be kept below a threshold temperature by failing to apply heat at that location, and a wax valve material can be mobilized from a storage area on a platform by heating and them allowed to flow under centripetal acceleration to a particularly "cold" site where a wax valve is desired. An advantage of wax valves in this regard is that the proper positioning an activation of resistive heater elements enables flexibility in choosing when and whether a particular microchannel, capillary or other fluidic component of the microsystems platforms of the invention is to be occluded by a wax sacrificial valve.

In particularly preferred embodiments, the sacrificial valves of the invention comprise a cross-linked polymer that displays thermal recover, most preferably a cross-linked, prestressed, semicrystalline polymer; an example of a commercially available embodiment of such a polymer is heat recoverable tubing (#FP301H, 3M Co., Minneapolis, MN). Using these materials, at a temperature less than the "melting"

temperature ($T_m$), the polymer occludes a microchannel, capillary or other fluidic component of the microsystems platforms of the invention. At a temperature greater than $T_m$, however, the polymer reverts to its pre-stressed dimensions by shrinking. Such shrinking is accompanied by release of the occlusion from the microchannel, capillary or other fluidic component of the microsystems platforms of the invention. Such embodiments are particularly~~particular~~ preferred because the polymer remains *in situ* and does not recrystallize or otherwise re-occlude the microchannel, capillary or other fluidic component of the microsystems platforms of the invention. Also, such embodiments do not require the more extensive manipulation in preparing the platforms of the invention that wax valves require.

**Page 74, line 9 to page 75, line 4**

A second general scheme for performing assays on the microsystems platforms of the invention involve miniaturized versions of affinity chromatography column separations, wherein the analyte specifically binds to a material in a chamber or on a surface, most preferably a derivatized surface, of the platform, or is bound to a material such as a bead, chromatography resin, or membrane on the surface of the platform, so that the remainder of the fluid sample can be washed from the affinity matrix and the analyte separated thereby. In certain preferred embodiments, the analyte is detected indirectly, wherein the biological fluid sample is interrogated after passage of the sample over the ~~chromatograchy~~ chromatography matrix. Such detection methods can be subtractive, wherein the interrogated optical property of the biological fluid sample after passage over the chromatography matrix is compared with the same property of a portion of the sample that has not been passed over the matrix; or directly, wherein the analyte is dissociated from the chromatography matrix (either non-specifically, using *for example* a salt or dielectric gradient, or specifically, using a binding competitor that displaces the analyte from the chromatography matrix).

**Page 76, line 18 to page 77, line 8**

The components of the blood glucose assay were prepared as follows. Blood sample entry port chamber **101** having a depth in the platform surface of about 0.32cm and lateral dimensions of about 1cm was constructed on the platform, and designed to accommodate a volume of 100 μL. This entry port was fluidly connected with a metering capillary **102** having a square cross-sectional diameter of about 0.1cm deep x 0.5cm ~~wideand~~wide and proximal ends rounded with respect to entry port **101**; the length of this metering capillary array was sufficient to contain a total volume of about 16μL. The entry port was also fluidly connected with an overflow capillary **103** having a cross-sectional diameter of about 0.05 cm x 0.075cm and proximal ends rounded with respect to entry port **101**. The overflow capillary was fluidly connected with a two-layered overflow chamber **105** having a first depth in the platform of about 0.025cm and a second depth in the platform of about 0.25cm, greater than the depth of the overflow capillary **103**. Metering capillary **102** was fluidly connected to metered blood fluid chamber **104** having a depth in the platform surface of 1mm and greater than the depth of the metering capillary **102**. Each of the overflow and fluid chambers was also connected with air ports or air channels, such as **114**, that have dimensions of 0.025cm deep and permitted venting of air displaced by fluid movement on the platform. A capillary junction **115** that was .051cm deep was present in the air channel to prevent fluid flow into the air channel.

**Page 80, line 1 to page 82, line 27**

Construction of the disk embodiments of the platforms of the invention are as described in Example 1. The blood application and metering components and their dimensions and relationships to one another are identical to those described above, comprising sample entry port chamber ~~801~~901, metering capillary ~~802~~902, overflow capillary ~~803~~903, metered blood fluid chamber ~~804~~-904 and overflow chamber ~~805~~905. As in Example 1, each of the overflow and fluid chambers is also connected with air

ports or air channels, such as ~~814~~914, and capillary junction(s) ~~815~~915, that permit venting of air displaced by fluid movement on the platform.

Blood fluid chamber ~~804~~ 904 was fluidly connected to capillary ~~810~~ 910 that was about 0.25mm deep and had a cross-sectional diameter of about 0.25mm and was connected to sacrificial valve ~~811~~911. Sacrificial valve ~~811~~ 911 was further fluidly connected with capillary ~~812~~ 912 that was about 0.25mm deep and had a cross-sectional diameter of about 0.25mm. Capillary ~~812~~ 912 was further fluidly connected to mixing chamber ~~815~~ 915 that was about 0.25cm~~em~~ deep, had a cross-sectional diameter of about 1cm, and was positioned about 2.5cm from the center of rotation. Lysis buffer was loaded directly onto mixing chamber ~~815~~ 915 in this embodiment and did not use capillary ~~818~~ 918 or lysis buffer chamber ~~816~~ 916 as shown in the Figure. 45μL of lysis buffer was applied to the mixing chamber as a solution of 0.1% Triton X100 in 50mM Tris pH 9.5.

Mixing chamber ~~815~~ 915 was fluidly connected to capillary ~~817~~ 917 that was about 0.25mm deep and had a cross-sectional diameter of about 0.25mm, and was connected to secondary metering structure ~~819~~919. Secondary metering structure ~~819~~ 919 was about 0.1cm deep and was positioned about 3.5cm from the center of rotation. Secondary metering structure ~~819~~ 919 was constructed to comprise two sections. A metering section was arranged proximal to the entry position of capillary ~~817~~ 917 and was separated from an overflow section by a septum that extended from the distal wall of the structure to a position just short of the proximal wall of the structure. This arrangement produced a fluid connection between the first metering section having a volumetric capacity of about 6.4μL and the overflow section having an excess volumetric capacity of 90μL. The volumetric capacity of the overflow section was sufficient to accommodate the largest blood fluid volume applied to the disk.

Capillary ~~821~~ 921 was in fluid connection with secondary metering structure ~~819~~ 919 at the distal wall of the metering section. Capillary ~~821~~ 921 was about 0.15cm deep and had a cross-sectional diameter of about 0.05cm and was connected to boronate affinity matrix chamber ~~822~~922. Boronate affinity matrix chamber ~~822~~ 922 was about

0.15cm deep, had a cross-sectional diameter of about 0.3cm and was positioned about 4.8cm from the axis of rotation. Boronate affinity matrix chamber ~~822~~ 922 was filled with boronate-functionalized agarose beads having a mean diameter of about 60μm; the beads were maintained in the chamber ~~822~~ 922 using a porous frit ~~827~~927. Fluid flow through capillary ~~821~~921 was connected to capillary or sacrificial valve ~~823~~923. Boronate affinity matrix chamber ~~822~~ 922 was further comprised of a translucent window that permitted reflective spectrophotometry of the contents of the chamber.

Boronate affinity matrix chamber ~~822~~ 922 was further fluidly connected to capillary ~~828~~928. Capillary ~~828~~ 928 was about 0.25mm deep and had a cross-sectional diameter of about 0.25mm and was connected to column wash buffer reservoir ~~829~~929. Column wash buffer reservoir ~~829~~ 929 was about 0.25cm deep and had a cross-sectional diameter of about 2cm and was positioned about 3.6cm from the axis of rotation, more proximal to the axis of rotation than boronate affinity matrix chamber ~~822~~922. Column wash buffer reservoir ~~829~~ 929 comprises 290μL of column preparation buffer that was a solution of magnesium chloride, taurine, D,L-methionine, sodium hydroxide, antibiotics and stabilizers constituted according to the manufacturer's instructions (Isolab Inc. #SG-6220). Fluid flow through capillary ~~828~~ 928 was connected to capillary or sacrificial valve ~~836~~936.

Boronate affinity matrix chamber ~~822~~ 922 was further fluidly connected to capillary ~~832~~932. Capillary ~~832~~ 932 was about 0.5m deep and had a cross-sectional diameter of about 0.5mm and was connected to non-glycated hemoglobin read chamber ~~834~~934. Chamber ~~834~~ 934 was about 0.25cm deep and had a cross-sectional diameter of about 2cm and was positioned about 5cm from the axis of rotation and was further comprised of a translucent window that permitted reflective spectrophotometry of the contents of the chamber at 430nm.

As illustrated in Figure 11, in the use of this platform about a 6.4 μL volume of blood was applied to blood fluid chamber ~~804~~904, either directly or using the metering components of the platform described above. Blood flowing through capillary ~~810~~ 910 and lysis buffer contained in mixing chamber ~~815~~ 915 were mixed in the mixing

chamber. A 45μL volume of lysis buffer was mixed with the blood sample. Fluid flow within mixing chamber ~~815~~ 915 was turbulent, in contrast to fluid flow through capillaries ~~810~~ 910 or ~~818~~918, which was primarily laminar, so that mixing occurred predominantly in mixing chamber ~~815~~915. Fluid flow proceeded through channel ~~817~~ 917 and into secondary metering structure ~~819~~919. The mixture of lysis buffer and blood, comprising a lysed blood sample ~~841~~941, flowed at a rotational speed $f_?$ of about 750rpm into secondary metering structure ~~819~~ 919 with release of a sacrificial valve ~~853~~953. Lysed blood sample ~~841~~ 941 entered and filled the metering section of secondary metering structure ~~819~~919. Any additional lysed blood sample then emptied into the overflow chamber of secondary metering structure ~~819~~ 919 and filled the total hemoglobin read chamber. Most preferably, a sufficient volume of lysis buffer and blood sample was applied to the disc to fill at least the metering sections of secondary metering structure ~~819~~ 919 and the total hemoglobin read chamber.

After the lysed blood sample ~~841~~ 941 was completely transferred to secondary metering structure ~~819~~919, capillary or sacrificial valve ~~823~~ 923 was released, allowing the metered lysed blood sample from the metering section of secondary metering structure ~~819~~ 919 through capillary ~~821~~ 921 and into boronate affinity matrix ~~822~~922. Capillary or sacrificial valve ~~836~~ 936 was then released, allowing about 290μL of column wash buffer ~~843~~ 943 to flow at rotational speed $f_?$ through capillary ~~830~~ 930 and into boronate affinity matrix ~~822~~922. Continued or discontinuous rotation motivates column preparation buffer through boronate affinity matrix ~~822~~ 922 and into non-glycated hemoglobin read chamber ~~834~~934. The control sample read cuvettes 8- and 8~ were then then illuminated by light at a wavelength of 430nm and the blank reading, i.e. reflectance from cuvettes containing only buffer, was determined. The non-glycated hemoglobin read window was then illuminated by light at a wavelength of 430nm and the concentration of non-glycated hemoglobin in the sample that has eluted from the column was determined by reflectance spectroscopy. The total hemoglobin read window was also illuminated by light at a wavelength of 430nm and the concentration of total hemoglobin in the lysed sample was determined by reflectance spectroscopy.

The relative amount of non-glycated hemoglobin in the sample is determined by dividing the amount of hemoglobin obtained by illuminating the eluted fraction by the amount of hemoglobin obtained in the total fraction.

**Page 86, line 21 to page 87, line 4**

Boronate affinity matrix chamber **28** is further fluidly connected to capillary **37**. Capillary **37** is about 0.5mm deep and has a cross-sectional diameter of about 0.5mm and is connected to sample collection cuvette array **12**. Sample collection cuvette array **12** is about 0.25cm deep and has a cross-sectional diameter of about 2.1cm and is positioned about 5cm from the axis of rotation. Sample collection cuvette array **12** is separated into a multiplicity of individual chambers, each separated from one another by septa that extend from the distal wall of the cuvettes to a position adjacent to the proximal wall of the cuvettes, so that a fluid passage **50** is maintained between each of the cuvettes. The fluid passage **50** is formed by the back (proximal wall) of the sample collection cuvette array **12** and the row of septa separating each of the sections of the sample collection cuvettes **12**. Capillary 3733 is fluidly connected to sample collection cuvette array **12** at a position adjacent to the proximal wall of the array and directed to the cuvette most proximal to the boronate affinity matrix chamber **28**.

**Page 90, lines 9-17**

The assays were performed on a microsystems platform according to ~~Examine~~ Example 3 using a round reagent cuvette (**113**). 60-70μL of human whole blood was applied to the disk, and 15μL metered into the metering capillary. A glucose reagent pad obtained from LifeScan was used for performing the glucose determination. Optical absorbance readings were obtained at 430nm, 590nm and 660nm on the unreacted reagent pad as a control. Blood was released into the reaction chamber and optical data gathered at one-second intervals for about 1 min. Absorbance at 590nm was specific for the amount of glucose in the sample; absorbance at 430nm was specific for hemoglobin

in the sample; and absorbance at 660nm was used to detect non-specific background absorbance, as described above.

**Page 92, lines 4-13**

For glucose chromagen alone, Sample D, the Reflectance(430nm) is 16.1% of the Reflectance(590nm) [(0.131-(-0.0204))/(0.9576-0.152)]. For hemoglobin alone, Sample B, the Reflectance(590nm) is 60.5% of the Reflectance (430) [(0.2116-0.0152)/(0.3041-(-0.0204))]. For the combination of glucose and hemoglobin, Sample C, the Reflectance(430nm) is the sum of the Reflectance (430nm) due to the hemoglobin plus 16.1% of the Reflectance(590nm) due to the glucose chromagen; similarly, the Reflectance(590nm) is the sum of the Reflectance(590nm) due to the glucose chromagen plus 60.5% of the Reflectance(430nm) due to the hemoglobin. Using standard algebraic methods, the factor to be used was calculated to be:

**Page 93, lines 3-4**

Seven separate assays were performed on each of 2 ~~difference~~ different whole blood samples. These results are as follows: